

A new method is described for evaluating air filters with submicron aerosols. The method is unique in that the aerosols were viable, highly concentrated, and composed entirely of submicron particles (0.1 micron NMD). Tests were conducted to compare air filters in removing submicron T1 phage aerosols and bacterial aerosols of Bacillus subtilis var niger spores (1 micron NMD). Architects, engineers, and research investigators concerned with the control of submicron particles might consider filtration rather than other methods of air cleaning.

AIR FILTRATION OF SUBMICRON VIRUS AEROSOLS

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Introduction

FILTRATION has been used extensively for removing microbial particles from air. Information on filter performance for microbial aerosols has been concerned mainly with bacterial aerosols in the 1- to 5-micron particle size range.¹ There is a lack of critical information on filter performance for viral aerosols of a submicron particle size that can be attributed to the problems associated with the generation, sampling and particle sizing of submicron viral aerosols. Initial studies by Decker, et al., on the removal of bacteriophage from air by spun glass filter pads were conducted with an aerosol generator that produced aerosols in the 1- to 5-micron particle size range. The phage suspension used for aerosol production was not purified and the aerosol particles consisted mainly of the extraneous materials present in the suspension. The phage particles contributed little to the mass of the aerosols. Therefore, the filter efficiencies obtained were for virus particles with

their associated menstrea rather than aerosols of a submicron particle size.² However, with the advancement in aerosol technology, new technics for virus purification and the development of improved aerosol generators such as the Dautrebande D₃₀1, it is now possible to evaluate air filters with submicron viral aerosols.

Submicron aerosols have been used for filter evaluation but the work has been limited to inert aerosols produced from dilute solutions of dyes and salts.³ Such information does not satisfy those concerned with the control of airborne microbial contamination.

This paper reports on the evaluation of four commercially available ultra-high-efficiency air filter units and one type of high-efficiency glass fiber filter medium with submicron T1 bacteriophage aerosols having a number median diameter (NMD) of 0.1 micron and with aerosols of *Bacillus subtilis* var niger spores with a NMD of 1 micron. Methods for phage purification and for generating, sampling, and particle siz-

ing submicron phage aerosols are reported in detail in a previous publication.⁴ This paper, therefore, will cover only the broad aspects of these methods. Procedures for evaluation of filters with bacterial aerosols may be found in publications by Decker, et al.^{1,2,5,6}

Materials and Methods

A. Test Filters

Air filters from four commercial manufacturers were chosen for this study. The air filters, shown in Figure 1, included four ultrahigh-efficiency filter units, also termed high-efficiency particulate air filters (HEPA) or absolute filters, and one type of high-efficiency glass fiber filter medium. These filters represented some of the typical air filters in use at the Biological Center and were ordered through normal procurement channels

from the individual manufacturers. The filters were evaluated first with submicron aerosols of T1 bacteriophage and then with bacterial aerosols of *B. subtilis* var niger spores.

B. Filter Testing Apparatus

The filter testing apparatus is illustrated in Figure 2. The Dautrebande D₃₀1 aerosol generator was used to produce the submicron phage aerosols. The bacterial aerosols were generated with the Vaponefrin nebulizer (Vaponefrin Co., Metuchen, N. J.). After leaving the generator the aerosol passed into a 3-inch diameter duct where the aerosol was mixed with filtered room air, then passed through the test filter and into the post-filter duct and finally exhausted by a blower to the outside air. The system was under a slight negative pressure (0.4 inch of water) to prevent the escape

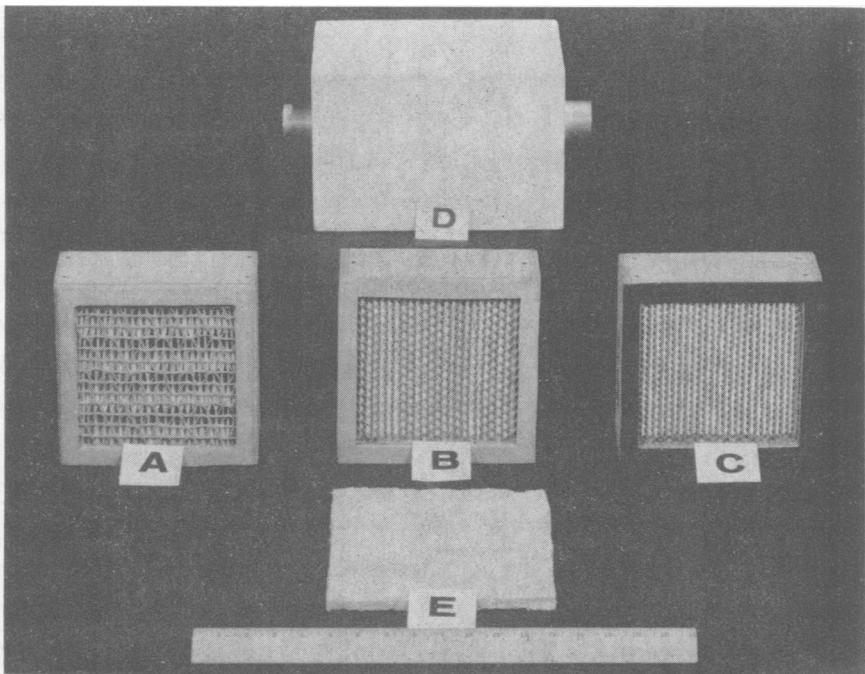


Figure 1—Four ultrahigh-efficiency filter units and one type of high-efficiency glass fiber filter medium.

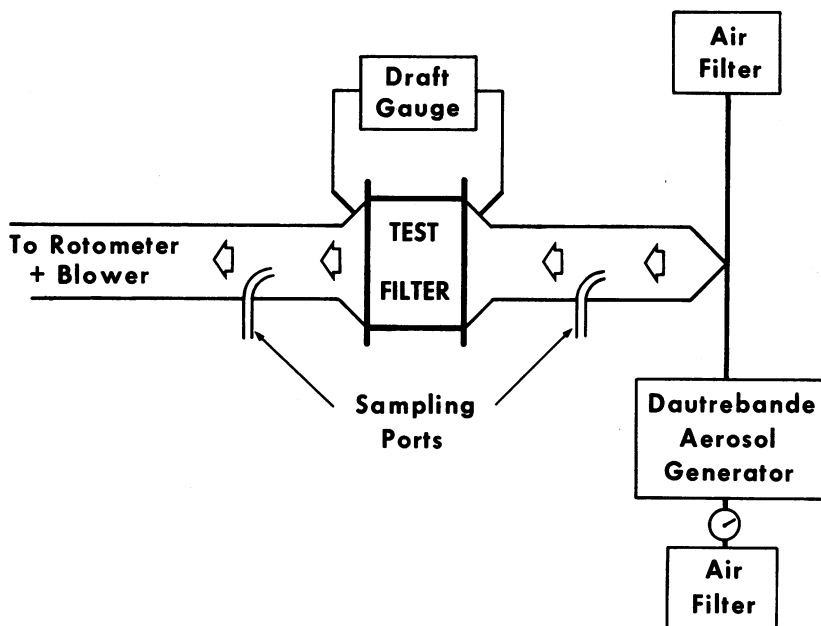


Figure 2—Filter testing apparatus.

of the test aerosol into the room. Air supplies for aerosol generation and mixing were filtered to assure clean particle-free air. This was confirmed by electron micrographs. Aerosol relative humidity and temperature measurements were obtained from wet and dry bulb thermometers attached to the duct. The resistance of the test filters was determined with a draft gauge.

C. Design of Experiments

Each ultrahigh-efficiency filter unit was tested three times with phage and then three times with *B. subtilis* spores. The high-efficiency glass fiber filter medium was also evaluated three times with each organism, but three different samples of the filter medium were used.

D. Phage Aerosol Tests

1. Phage Suspension

The virus used for the filter tests was T1 bacteriophage, one of the viruses

parasitic to *Escherichia coli*, strain B. Aqueous suspensions of purified T1 phage were prepared by concentrating and purifying large volumes of broth cultures by differential centrifugation and washing with distilled water. This thorough cleansing of the phage suspensions was necessary to produce aerosols of minimal particle size. An electron micrograph of T1 phage particles from an aqueous purified suspension prepared by the phosphotungstic acid negative staining method of Brenner and Horne⁷ is shown in Figure 3. The purified phage suspension, used for evaluating the filters, titered 2×10^{11} phage/ml and contained 1×10^{-4} g/ml of Dextran 2000 (Pharmacia, Uppsala, Sweden) for enhancing the biological aerosol stability.

2. Phage Aerosol Sampling

The all-glass impinger (AGI-4) (Ace Glass Co., Vineland, N. J.) with a backup filter of ultrahigh-efficiency filter paper was used to determine the phage aerosol concentrations before and after the test

filter. The AGI-4 was filled with 22 ml of Bacto nutrient broth containing 0.06 per cent Dow Corning Antifoam A (Dow Corning Corp., Midland, Mich.) and was operated at the maximum (approximately sonic) flow rate of 12.5 liters per minute. The backup filter, which collected the phage aerosol slipping through the impinger, was Type 6 and Type 5 Chemical Corps filter papers sealed in an in-line filter holder.⁸ Chemical Corps Type 6 filter paper (Hollingsworth & Vose Co., East Walpole, Mass.) is an ultrahigh-efficiency paper composed of Bolivian or African blue asbestos, esparto grass, and kraft fibers. The Type 5 filter paper is a lower efficiency paper of cellulose and asbestos fibers with a backing of cotton scrim. It was used only to support the more fragile Type 6 paper but was assayed with the Type 6 paper. Immediately after sampling, the filter papers were placed in 100 ml of Bacto nutrient

broth containing 0.1 per cent Tween 20 (Atlas Powder Co., Wilmington, Del.) and then shaken for 15 minutes on a mechanical shaker to disintegrate the paper to pulp.

Flow rates of sampler air were calibrated with a wet test meter. The all-glass impingers were also calibrated for clearance of orifice to flask bottom and only those with a clearance of 4 mm were selected for these tests.

The phage aerosol particle size was determined by collecting a prefilter aerosol sample on an electron microscope grid mounted in an electrostatic precipitator.^{9,10} The grid was then examined in an electron microscope at a magnification of 7,000 and the aerosol particles were photographed. Typical results are shown in Figures 4 and 5. Figure 4 is a graph of a phage aerosol at 19 per cent relative humidity showing the cumulative percentages by number and by mass

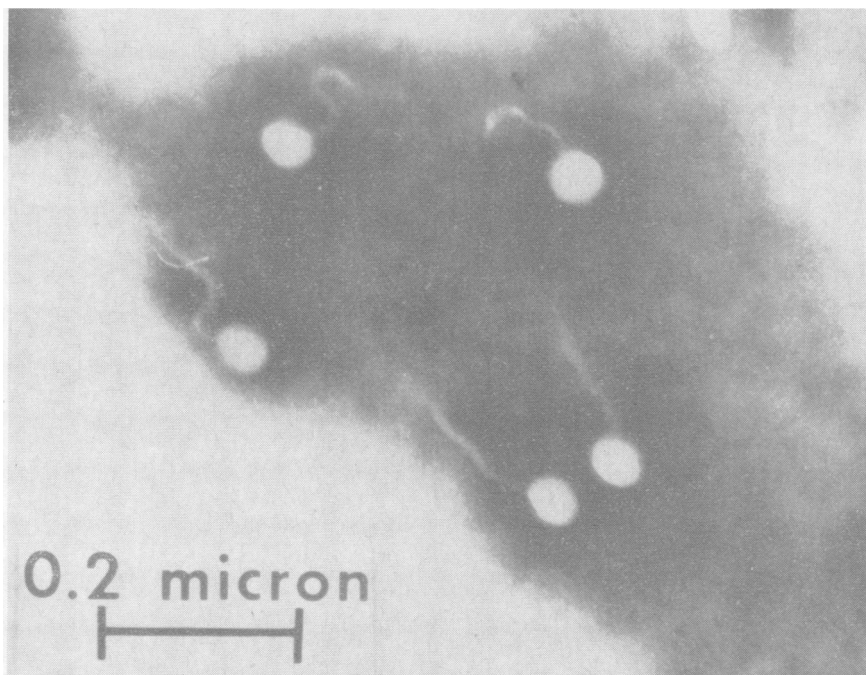


Figure 3—Electron micrograph of a purified T1 phage suspension negatively stained with phosphotungstic acid.

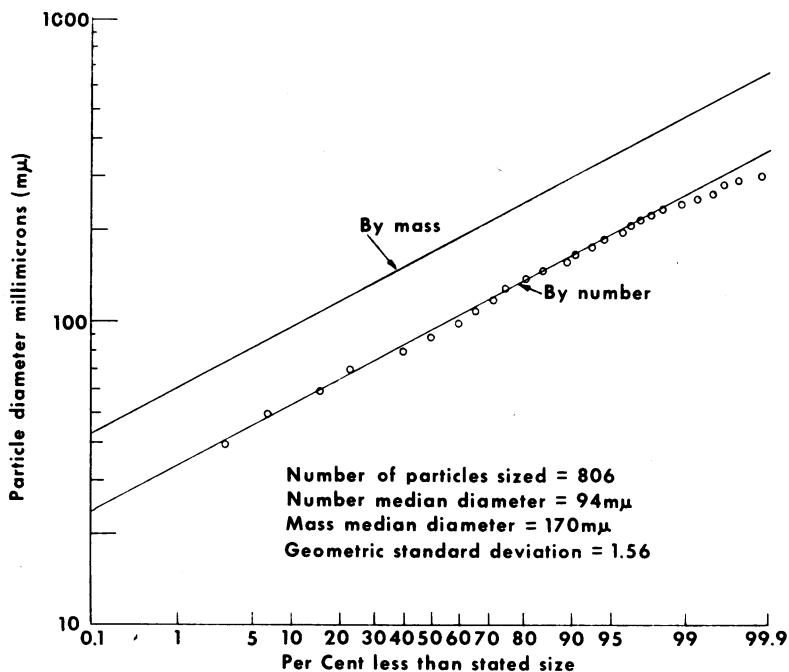


Figure 4—Size distribution of T1 phage aerosol at 19 per cent relative humidity dispersed with Dautrebande $D_{30}1$ aerosol generator.

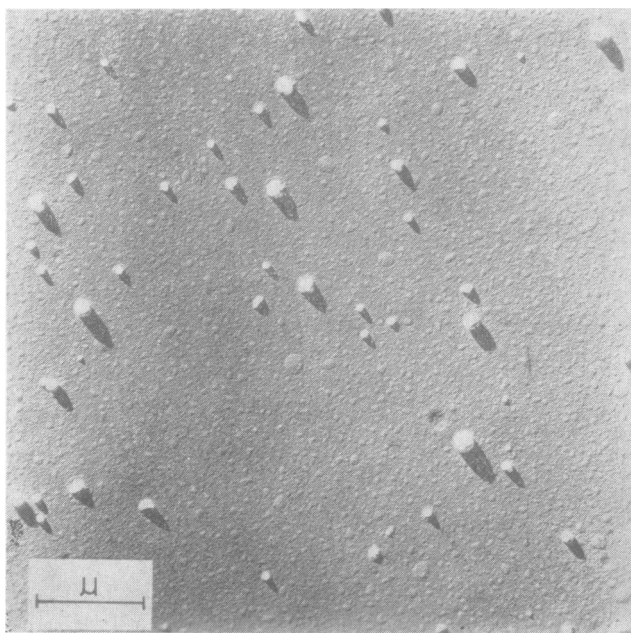


Figure 5—Electron micrograph of T1 phage aerosol dispersed with Dautrebande $D_{30}1$ aerosol generator.

plotted against particle diameter on log probability paper. The median diameters (50 per cent intercepts) are 94 $m\mu$ NMD and 170 $m\mu$ MMD. Figure 5 is an electron micrograph of this aerosol.

The schedule followed for each phage aerosol test consisted of filling the Dautrebande generator with 5 ml of the phage suspension and disseminating the aerosol continuously for 5 minutes. The generator was operated at 17.5 psig, which resulted in an airflow through the generator of 18.8 liters per minute. The aerosol samplers were turned on before the Dautrebande generator and were turned off one minute after the generator was turned off. Two impingers with their backup filters were used at both the pre- and postfilter sampling stations. The samples were pooled and assayed by making duplicate serial dilutions in nutrient broth and plating each dilution in triplicate using an agar layer method as described by Harstad.⁴ Penetration of the aerosol through the test filters was calculated from the total sampler (impinger + backup filter) recoveries, i.e., backup filter recovery was added to impinger recovery.

Slippage of the phage aerosol through the impinger was also calculated from the impinger and backup filter recoveries as given by:

$$\text{Slippage} = \frac{\text{Backup filter}}{\text{Backup filter} + \text{impinger}}$$

Slippage was 23 per cent for the prefilter impingers and 25 per cent for the postfilter impingers.

An earlier study with radioactive submicron phage aerosols⁴ revealed that impingers (AGI-4) allow considerable slippage but are less destructive to phage than Type 6 filter paper, which allows almost no slippage. Therefore both samplers were used for the present study; an impinger used first to collect the bulk of the aerosol followed by a backup sampler of Type 6 filter paper to collect the phage aerosol slipping through the

impinger. Obviously, the total sampler (impinger + backup) recoveries would have been higher than the observed recoveries if there were no destruction (death) of phage by the backup filter sampler. However, the test filter penetration results (Table 1) were not affected because penetration is the ratio of the pre- and postfilter sampler recoveries and prefilter impinger slippage was virtually the same as postfilter impinger slippage.

E. Bacterial Aerosol Tests

After the phage aerosol tests, the test filters were evaluated with aerosols of *B. subtilis* var niger spores, which are highly resistant and therefore ideal test organisms. The aerosols were produced from an aqueous suspension of heat-shocked spores titering 4×10^9 spores per ml with a Vaponefrin nebulizer operated at 10 psig, which resulted in an air flow of 6 liters per minute through the nebulizer. Cotton collectors, operated at 10 liters per minute for a 10-minute sampling period, were used as the pre- and postfilter samplers. Immediately after sampling, the cotton was placed in 100-ml water blanks and shaken for 15 minutes. The samples were then assayed on pour plates with Bacto tryptose agar after making the appropriate serial dilutions in distilled water. Colonies were counted after 24-hour incubation at 37°. *C. B. subtilis* aerosols were sized in two ways: (a) electron microscopy of aerosol samples collected with the electrostatic precipitator, and (b) phase microscopy of aerosol samples collected on membrane filters. Both of these methods revealed that *B. subtilis* aerosols were composed mainly of single spores and had a NMD of 1 micron.

Results and Discussion

The penetration of phage aerosols and bacterial aerosols through air filters from

Table 1—Penetration of T1 phage^a and bacterial aerosols^b through commercial air filters

Filter type	Description	Test number	Relative humidity %	Test air flow	Filter resistance in. water	Penetration		
						T1 Phage ^c %	Bacterial spores ^d %	DOP ^e %
Ultra-high-efficiency	Glass micro-fibers, waterproofed, plastic base adhesive, 35 cfm rated capacity 8" × 8" × 3 5/16"	1	15	25	1.04	3.2 × 10 ⁻³	8.7 × 10 ⁻⁵	0.011
		2	to	cfm		4.3 × 10 ⁻³	9.6 × 10 ⁻⁵	
		3	20			4.3 × 10 ⁻³	1.4 × 10 ⁻⁴	
		Mean				3.9 × 10 ⁻³	1.1 × 10 ⁻⁴	
Ultra-high-efficiency	Glass asbestos fibers with organic binder, neoprene type sealer, 30 cfm rated capacity 8" × 8" × 3 1/16"	1	15	25	0.69	1.2 × 10 ⁻³	8.4 × 10 ⁻⁵	0.02
		2	to	cfm		6.0 × 10 ⁻⁴	6.1 × 10 ⁻⁵	
		3	20			7.6 × 10 ⁻⁴	7.2 × 10 ⁻⁵	
		Mean				8.5 × 10 ⁻⁴	7.2 × 10 ⁻⁵	
Ultra-high-efficiency	All-glass fibers with no organic binder, rubber base type sealer, 30 cfm rated capacity 8" × 8" × 3 1/16"	1	20	25	0.53	4.6 × 10 ⁻³	4.0 × 10 ⁻⁴	0.006
		2	to	cfm		3.9 × 10 ⁻³	1.7 × 10 ⁻⁴	
		3	25			4.7 × 10 ⁻³	2.8 × 10 ⁻⁴	
		Mean				4.4 × 10 ⁻³	2.8 × 10 ⁻⁴	
Ultra-high-efficiency	All-glass fibers with no organic binder, rubber base type sealer, 22 cfm rated capacity 8" × 8" × 12"	1	15	22	0.75	1.1 × 10 ⁻³	1.9 × 10 ⁻³	0.002
		2	to	cfm		1.0 × 10 ⁻³	2.2 × 10 ⁻³	
		3	20			9.9 × 10 ⁻⁴	2.8 × 10 ⁻³	
		Mean				1.0 × 10 ⁻³	2.3 × 10 ⁻³	
Over-all mean for ultrahigh-efficiency filter units						3 × 10 ⁻³	7 × 10 ⁻⁴	
High-efficiency	0.5" thick fiber-glass pads containing 1.25μ diameter glass fibers	1	40	20 ft	0.50	1.8	0.23	
		2	to	per	0.50	2.0	0.26	
		3	45	min ^f	0.51	1.9	0.50	
		Mean				1.9	0.33	

^a T1 phage aerosol number median diameter (NMD)—0.1 micron.^b B. subtilis var niger spore aerosol NMD—1 micron.^c Prefilter total sampler (Impinger+backup filter) recovery—10⁶ phage/liter.^d Prefilter cotton collector recovery—10⁵ spores/liter.^e DOP penetration as stamped on filter unit by manufacturer.^f Face velocity (1.5 cfm through 3 1/2 in. diameter filter pads).

four commercial manufacturers is compared in Table 1. Dioctyl phthalate (DOP) penetration, obtained from the manufacturers, is also shown.

Over-all mean phage aerosol penetration through ultrahigh-efficiency filter units was 3×10^{-3} per cent. The comparable value for the bacterial aerosols of *B. subtilis* var niger spores was 7×10^{-4} per cent. Phage penetration through the ultrahigh-efficiency filters is remarkably low and these filters will provide excellent protection against submicron virus aerosols. Design engineers concerned with the construction and remodeling of biomedical research facilities in which infectious microorganisms are handled might consider ultrahigh-efficiency filters instead of the more expensive air incinerators used in several laboratories. Only with a high concentration of pathogenic aerosols should serious consideration be given to some of the more complex and more efficient air purification systems.

The high-efficiency glass fiber filter material exhibited a mean penetration of 1.9 per cent for the submicron T1 phage aerosols and 0.33 per cent for the *B. subtilis* var niger aerosols. This is considered excellent for this category of filter and its application as a roughing filter or even as a final filter in certain laboratories might be practical.

Dioctyl phthalate (DOP) penetration shown in the table was stamped on each filter unit by the manufacturer and was verified by the DOP test apparatus at another government installation. The correlations among DOP, phage, and bacterial penetration were not good. The DOP aerosol is a homogeneous fog of submicron particles that are uniform spheres of about 0.3 micron diameter.

Phage and bacterial aerosols are heterogeneous in size and shape. The DOP method is useful for checking for possible major leaks resulting from damaged filters or inadequate sealing around the filters. Such leaks can be quickly detected by the DOP method and are usually in good agreement with bacterial leakage. However, with filters of ultrahigh-efficiency such as those tested in this study, the DOP readings are near the bottom of the instrument scale, where background or noise level of the instrument precludes accurate measurement.

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